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ELECTROANTENNOGRAM RESPONSES OF THE MEDITERRANEAN FRUIT FLY, *Ceratitis capitata*, TO A SPECTRUM OF PLANT VOLATILES

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Abstract—Electroantennograms (EAGs) were recorded from unmated, laboratory-reared, male and female *Ceratitis capitata* (medfly) in response to a range of C_1 and C_2 to C_{12} carbon chain-length aliphatic alcohols, aldehydes, acetates, and acids, and lactones, some of which are known volatiles from leaves and fruits. A large degree of EAG response uniformity between the sexes was observed, with only eight of the 70 compounds tested eliciting significantly larger amplitude EAG responses from female than male antennae. In general, for the five functional-group series tested, aldehydes and alcohols elicited greater responses than acetates, lactones, and acids. The unsaturated alcohols, aldehydes, acetates, and acids elicited equal or larger amplitude EAG responses than their comparable saturated compounds. For four of the functional-group series tested, the EAG response amplitude was significantly greater for a particular carbon chain length, with responsiveness to primary alcohols and aldehydes peaking at C_6, acids peaking at C_{5-6}, and acetates peaking at both C_5 and C_8. The EAG responses to both the 2- and 3-position monoenic alcohols peaked at C_6 and C_8, while the secondary alcohols peaked at C_7. The greatest EAG responses of all compounds tested were elicited by monoenic C_6 alcohols and aldehydes that are constituents of the "general green-leaf odor" that emanates from most plants. The potential adaptive benefit of selective sensitivity to green-leaf volatiles is discussed in regard to foraging behavior of medflies.

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Key Words—Diptera, Tephritidae, Mediterranean fruit fly, *Ceratitis capitata*, plant volatiles, fruit volatiles, green-leaf volatiles, olfaction, electrophysiology, electroantennogram.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (medfly), is a polyphagous pest worldwide, attacking over 250 varieties of fruits, nuts, and vegetables (Hagen et al., 1981). It is widely assumed that medflies and other tephritid fruit flies use olfactory cues, in addition to visual cues (Cytrynowicz et al., 1984; Nakagawa et al., 1978; Prokopy and Roitberg, 1984; Prokopy and Economopoulos, 1976), to seek and assess habitat, food, and ovipositional resources (Prokopy and Roitberg, 1984). Such a phenomenon has been observed in studies of the behavior of the apple maggot, *Rhagoletis pomonella* (Walsh), in response to fruit model traps emanating apple volatiles (Prokopy et al., 1973; Reissig et al., 1982). However, there is little behavioral evidence (Bateman, 1972; Féron, 1962; Keiser et al., 1975) to substantiate this assumption of olfactory orientation to plant volatiles by *C. capitata*.

The olfactory fragrance of a plant and, in particular, a ripening fruit, is a complex blend of usually over a hundred(s) detectable volatile compounds, possessing various functional groups and ranging in structure from simple, short, straight carbon chains to complex multiring sesquiterpenes (e.g., Buttery, 1981; Van Straten and Maarse, 1983) (Table 1). Although the host-plant range of medflies is extremely broad and diverse, most of the host-plant and fruit odors will share some constituents from various classes of volatiles, e.g., aliphatic alcohols, aldehydes, acetates and acids, and monoterpenes, sesquiterpenes, and lactones (Buttery, 1981; Van Straten and Maarse, 1983; Visser et al., 1979) (Table 1).

Little is known about the neurophysiological reception of plant volatiles by Diptera in general, let alone *C. capitata* and other tephritid species. To date, only four electrophysiological studies have been reported on tephritid fruit flies. Recently, Van Der Pers et al. (1984) utilized the electroantennogram (EAG) technique to study the responsiveness and sensitivity of male and female *Dacus oleae* (Gmelin) (olive fruit fly) to six components of its putative pheromones and five chemical analogs, some of which are commonly occurring plant volatiles. An EAG study on tephritids that tested "generally occurring fruit volatiles" was briefly reported by Guerin et al. (1983a). They recorded EAGs from female antennae of three species, *D. oleae, Rhagoletis cerasi* (L.) (cherry fruit fly), and *C. capitata*. Although 30 volatiles were tested (series of aliphatic C_5 to C_{12} terminal position alcohols, aldehydes, and esters), only heptanal, octanal, nonanal, and (*E*)-2-nonenal were reported to elicit the highest amplitude

TABLE 1. SOURCE AND PURITY OF CHEMICALS USED IN ELECTROPHYSIOLOGICAL STUDIES AND THEIR PRESENCE IN A VARIETY OF HOST FRUITS OF *Ceratitis capitata*

Compound	Chemical purity (%)[a]	Source[b]	Presence in fruit of			
			Citrus[c,d]	Papaya[e]	Passion fruit[d]	Peach[d]
Aliphatic alcohols						
Ethanol	100.0	A	+	+	+	+
Propan-1-ol	95.0	B	+	+	+	
2-Propen-1-ol	99.0	C				
Butan-1-ol	100.0	D	+	+	+	+
(*E*)-2-Buten-1-ol	92.3	B				
3-Buten-1-ol	99.1	D				
Pentan-1-ol	99.5	D	+	+	+	+
(*E*)-3-Penten-1-ol	97.0	C				
Hexan-1-ol	100.0	D	+	+	+	+
(*E*)-2-Hexen-1-ol	95.9	D	+	+		+
(*Z*)-2-Hexen-1-ol	99.5	D				
(*E*)-3-Hexen-1-ol	100.0	D			+	
(*Z*)-3-Hexen-1-ol	99.9	D	+	+	+	
Heptan-1-ol	98.0	D	+	+	+	
(±)-Heptan-2-ol	99.2	D			+	
Octan-1-ol	100.0	D	+	+	+	+
(*E*)-2-Octen-1-ol	92.0	C				
(*E*)-3-Octen-1-ol	84.7	C			+	
(±)-1-Octen-3-ol	99.5	D	+			
Nonan-1-ol	100.0	D	+			
(±)-Nonan-2-ol	98.8	D	+		+	
Decan-1-ol	99.0	D	+			
Undecan-1-ol	96.5	B	+			
(±)-Undecan-2-ol	98.0	E				
Dodecan-1-ol	96.9	F	+			+

TABLE 1. (Continued)

Compound	Chemical purity (%)[a]	Source[b]	Presence in fruit of			
			Citrus[c,d]	Papaya[e]	Passion fruit[d]	Peach[d]
Aliphatic aldehydes						
Propanal	54.0	B				
Butanal	67.0	B	+			+
(*E*)-2-Butenal	93.9	F				
Pentanal	99.0	D	+			
Hexanal	98.3	D	+			+
(*E*)-2-Hexenal	98.6	D	+			+
Heptanal	98.1	D	+			+
Octanal	95.5	D	+			
Nonal	91.1	G	+			+
Decanal	97.9	D	+			
Undecanal	91.0	D	+			
Dodecanal	89.5	O	+			
Aliphatic acids						
Formic	88.0	H				+
Acetic	99.9	I	+		+	+
Propanoic	99.8	J	+			
2-Propenoic	95.0	B				
Butanoic	99.0	J	+		+	+
Pentanoic	99+	K	+		+	+
Hexanoic	98.2	D	+		+	+
(*E*)-2-Hexenoic	99.0	D			+	+
Heptanoic	98.2	D	+		+	
Octanoic	100.0	D	+		+	+
Nonanoic	94.1	D	+		+	

Decanoic	99+	D	+		+	+
Dodecanoic	99+	F	+		+	
Aliphatic esters						
Ethyl acetate	99+	H	+	+	+	+
Propyl acetate	98.0	F		+		
Butyl acetate	98.0	B	+	+	+	+
Pentyl acetate	37.0	O	+	+		+
Hexyl acetate	92.0	O		+	+	+
(*E*)-2-Hexenyl acetate	99.7	L				+
Heptyl acetate	97.0	B				
Octyl acetate	98.4	O	+		+	
Nonyl acetate	97.0	C	+			
Decyl acetate	95.0	C	+			
Lactones						
gamma-Butyrolactone	99+	M			+	
gamma-Pentalactone	96.6	D				+
gamma-Hexalactone	98.0	C		+	+	+
gamma-Heptalactone	88.2	M		+	+	+
gamma-Octalactone	95.0	D		+	+	+
gamma-Nonalactone	88.6	N		+	+	+
gamma-Decalactone	97.0	D		+	+	+
gamma-Undecalactone	97.1	N			+	+
mixture of C_8–C_{12} delta-lactones	90+	P		+	+	+

[a] Capillary GLC analysis (12.5-m × 0.2-mm methyl silicone cross-linked column) at USDA-ARS-WRRC, Albany, California.

[b] A, U.S. Industrial Chemicals; B, Eastman Kodak Co.; C, synthesized at USDA-ARS-WRRC, Albany, California; D, Aldrich Chemical Co.; E, Fluka Chemical Co.; F, Chem Service, Inc.; G, Fritzsche, Dodge and Olcott, Inc.; H, Fisher Scientific Co.; I, J.T. Baker Chemical Co.; J, Mallinckrodt Chemical Co.; K, Sigma Chemical Co.; L, CTS Organics; M, K&K Laboratories; N, Norda Chemical Co.; O, source presently unknown, from file at USDA-ARS-WRRC, Albany, California; P, Oril Chemical Co.

[c] Kefford and Chandler (1970).

[d] Van Straten and Maarse (1983).

[e] Flath and Forrey (1977).

EAGs. In addition, Fein et al. (1982) recorded the EAGs of *R. pomenella* to a group of seven esters they identified from apple odor.

The purpose of this initial study was to investigate, by means of EAGs, peripheral olfactory selectivity of adult *C. capitata* to commonly occurring classes of host-plant and fruit volatiles, including the ubiquitous "general green-leaf volatiles" (Visser et al., 1979). The volatiles tested ranged from C_1 or C_2 to C_{12} saturated and unsaturated aliphatic alcohols, aldehydes, acids, acetates, and a series of lactones (Table 1). This research was further intended to provide a basis for studies of single-cell responses by surveying and assessing the degree that the antennal olfactory system of medflies is receptive to classes and/or particular plant/fruit volatiles.

METHODS AND MATERIALS

Insects. Pupae of *C. capitata* were obtained from a laboratory colony, periodically infused with feral flies, maintained at the USDA, Tropical Fruit and Vegetable Research Laboratory, Honolulu, Hawaii. Upon their arrival, pupae were segregated by sex and placed in separate cages. After eclosion, adult flies were provided sucrose cubes, hydrolyzed protein, and water until they were tested two to five days after emergence.

Olfactory Stimuli. Table 1 lists the compounds tested, their supply sources, and purities. The compounds were dissolved in spectrometric grade hexane (that was additionally distilled and treated with an antioxidant) at a rate of one part test compound and nine parts hexane solvent, forming 10% volume per volume solutions. From these solutions, test cartridges were produced for each compound by pipetting 1-μl aliquots onto separate 1 × 2-cm pieces of fluted, glass-fiber filter paper, which were then inserted into individual Pasteur pipets. Before or during each experiment, new cartridges were loaded with compound, individually sealed in zip-lock plastic bags, placed in a freezer (−4°C), and then transferred to a fume hood just prior to testing.

Electrophysiological Recording Technique. Electroantennogram (EAG) techniques used here are a modification of previous techniques utilizing glass capillary Ag–AgCl electrodes filled with insect saline (Schneider, 1957a; Light, 1983). Intact flies were immobilized by a yoke in a Plexiglas block. The recording electrode was inserted into the distal region of the terminal antennal segment or funiculus, while the indifferent electrode was positioned into the hemocoel of the cranial cavity. The signal was amplified 100× by a Grass P-16 microelectrode amplifier (Quincy, Massachusetts) and viewed on either an analog (Tektronix 5113, Beaverton, Oregon) or digital (Nicolet 4094, Madison, Wisconsin) storage oscilloscope. EAG deflections were measured directly from the stored screen image or from either photographs (Tektronix C5-A camera, Polaroid-type 667 film) or digital graphs from an *x–y* plotter (Hewlett Packard 7475A, Sunnyvale, California).

Odor Delivery. The odor delivery system and stimulation technique were essentially the same as that described by Light (1983). A constant flow (1.0 liter/min) of charcoal-filtered and humidified compressed air was passed-over the antenna through a disposable nozzle (automatic pipet tip, Centaur Chemical, Stamford, Connecticut) positioned ca. 1 cm from the antenna. When activated by a timing circuit, a three-way solenoid valve diverted the purified air through the stimulus cartridge where evaporating volatiles were picked up and carried into the nozzle and then onto the antenna. Stimulation duration was 1.0 sec. Because of the variation in volatility of test compounds, only relative comparisons can be made between the odorous stimuli.

Experimental Procedure. For each stimulus, EAGs were recorded from at least five flies of each sex. "Control" stimulations (using filter papers either untreated or impregnated with 1 μl of the hexane solvent) and "standard" stimulations (using fresh cartridges impregnated with 1 μl of 1% hexan-1-ol) were interspersed at approximately every fifth to tenth stimulation.

EAGs to test compounds were evaluated by measuring the maximum amplitude of negative deflection ($-$mV) elicited by a given stimulus and then subtracting the amplitude of the response to the preceding control. The millivolt responses to all compounds were converted to percentage values of the response to the accompanying 1% hexan-1-ol standard, as used in other EAG studies on insect olfaction (Dickens, 1984; Dickens and Boldt, 1985; Light, unpublished). This conversion or normalization of each response to a percentage of standard response allowed for comparison of responses within an individual and among individuals (Payne, 1975). Furthermore, this procedure minimizes and the observed variability in (1) absolute responsiveness among preparations, (2) order of presentation of compounds, and (3) the time-dependent variability in antennal responsiveness (Light, 1983; Dickens, 1984). Mean responses were compared using a t test and the nonparametric Mann-Whitney test (Snedecor and Cochran, 1967). Each stimulation was followed by an interval of ca. 90 sec of clean air. This interval was adequate for recovery of the EAG amplitude, as demonstrated by the measured percent recoveries of both sexes to shorter, 60-sec intervals between two separate 10% stimulations of hexan-1-ol (100.6% $\pm$ 1.8% for males and 99.3% $\pm$ 2.1% for females).

RESULTS

Selectivity

In general, the following EAG results suggest significant differences in the size of acceptor populations for the various odorants and/or odorant classes examined. Although slight differences between males and females in the magnitude of their EAG responses to each test odorant was found, in only a few cases were the differences significant.

The mean responses of *C. capitata* antennae to the hexan-1-ol standard (1 μl of 1% v/v hexane) were not significantly different between males and females, with mean EAGs of -1.09 mV (SE = 0.08 mV) and -1.14 mV (SE = 0.11 mV) for 15 males and 13 females, respectively.

Aliphatic Alcohols. For the series of saturated primary alcohols tested, responses of both male and female medflies peaked at hexan-1-ol and declined as carbon chain lengths either increased and decreased from six (Figure 1). Only for undecan-1-ol were EAG magnitudes found to be significantly different between the sexes, with female antennae more responsive than male antennae ($P < 0.05$).

In general, for both of the limited series of *E* geometric configurations of the 2- and 3-unsaturated primary alcohols tested, antennal responsiveness of both sexes increased as chain length increased from three to eight carbons (Figure 2). The only exception found was of female antennae being slightly, but not significantly, more responsive to (*E*)-2-hexen-1-ol than to (*E*)-2-octen-1-ol. (*E*)-2-Buten-1-ol elicited significantly greater ($P < 0.05$) EAGs from females than males.

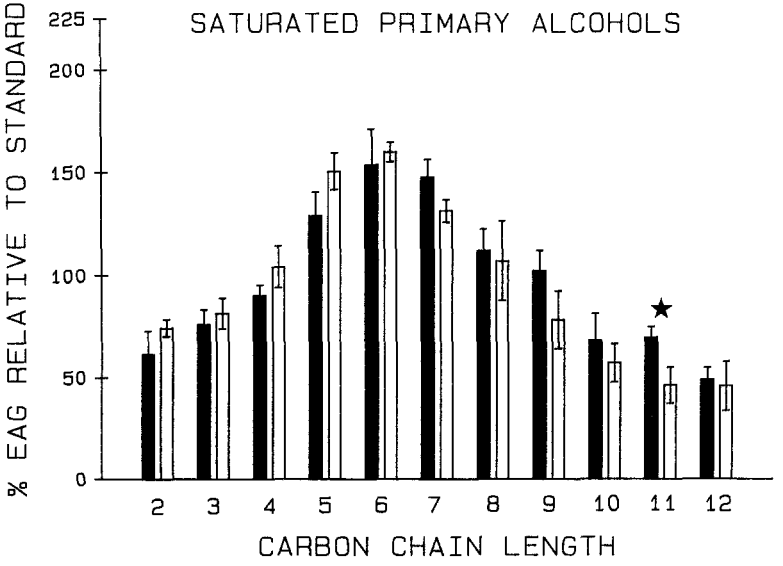

FIG. 1. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of saturated primary alcohols of various carbon chain lengths. Vertical lines represent standard errors, $N = 5$, a 100% response is approx. -1.1 mV, and stars represent significant differences in responsiveness between the sexes.

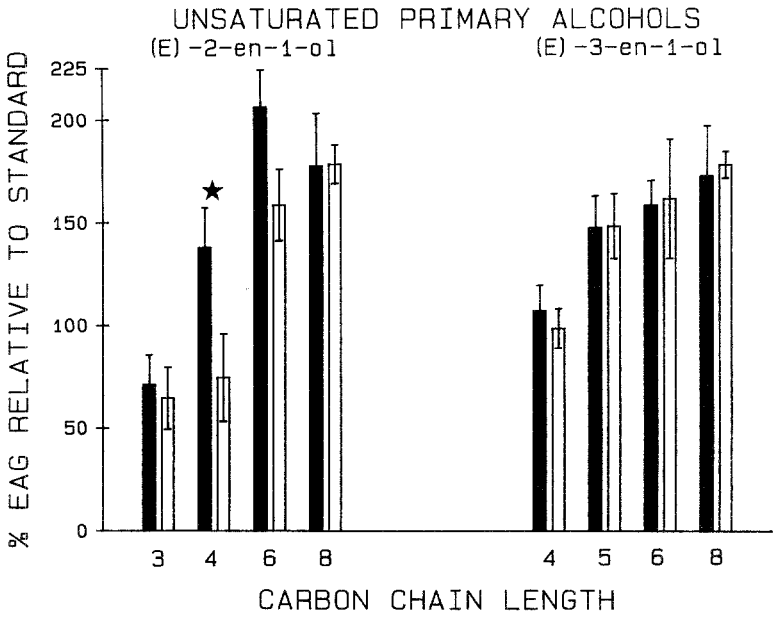

FIG. 2. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of unsaturated (*E*)-2- and (*E*)-3- primary alcohols of various carbon chain lengths. See Figure 1 legend for further information.

In two tests where *E* and *Z* geometric isomers of both hex-2-en-1-ol and hex-3-en-1-ol were compared, no significant differences were found between either of the hexenol isomers (Figure 3). Furthermore, there were no significant differences for either sex between the commonly tested 2- and 3-position unsaturated alcohols (i.e., the butenols and octenols, Figure 2) except for the hexenols (Figure 3). For males and females, both the (*Z*)- and (*E*)-hex-2-en-1-ol isomers elicited significantly ($P < 0.05$) greater EAGs than their hex-3-en-1-ol isomeric counterparts.

For the limited series of racemic saturated secondary alcohols tested, both male and female antennae were significantly less stimulated as chain length increased from seven to 11 carbons (Figure 4). EAGs of females to the secondary alcohols were slightly greater than males and were significantly greater ($P < 0.05$) for (±)-heptan-2-ol. The responses to the only 3-position and unsaturated secondary alcohol tested, (±)-1-octen-3-ol, were intermediate to the responses elicited by (±)-heptan-2-ol and (±)-nonan-2-ol. As with (±)-hepan-2-ol, female antennae were more responsive than male antennae to (±)-1-octen-3-ol.

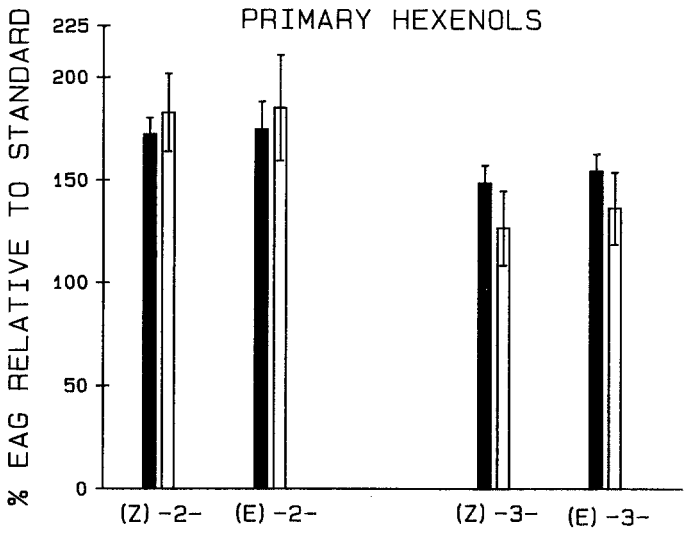

FIG. 3. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of primary hexenols. See Figure 1 legend for further information.

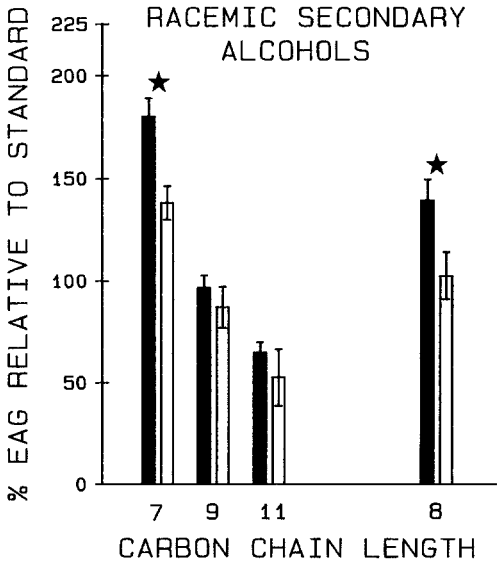

FIG. 4. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of racemic secondary alcohols of various carbon chain lengths, either saturated [(±)-heptan-2-ol, (±)-nonan-2-ol, and (±)-undecan-2-ol] or unsaturated [(±)-1-octen-3-ol]. See Figure 1 legend for further information.

Aliphatic Aldehydes. Both male and female antennae were significantly more responsive to the six- to 10-carbon chain saturated aldehydes than to either the lower or higher chain-length aldehydes (with the exception of dodecanal for females) (Figure 5). There was generally a stepwise progressive decrease in antennal responsiveness to aldehydes as chain length increased from hexanal to undecanal, but there was a significant upturn in responsiveness from undecanal to dodecanal. Among the saturated aldehydes tested, only hexanal elicited significantly different responses ($P < 0.05$) between the sexes, with female antennae more responsive than male antennae. Both of the unsaturated aldehydes tested, (*E*)-2-butenal and (*E*)-2-hexanal, elicited significantly greater ($P < 0.01$) EAGs than their saturated aldehyde counterparts (Figure 5).

Aliphatic Acids. The response magnitude rose significantly for both pentanoic and hexanoic acids over the lower, relatively level responses to both increasing and decreasing chain lengths of the other saturated acids (Figure 6). The inclusion of a double bond slightly, but not significantly, increased EAG responsiveness to both 2-propenoic and (*E*)-2-hexenoic acids over their respective saturated acids (Table 2 and Figure 6).

Aliphatic Acetates. The series of saturated acetates elicited EAGs that

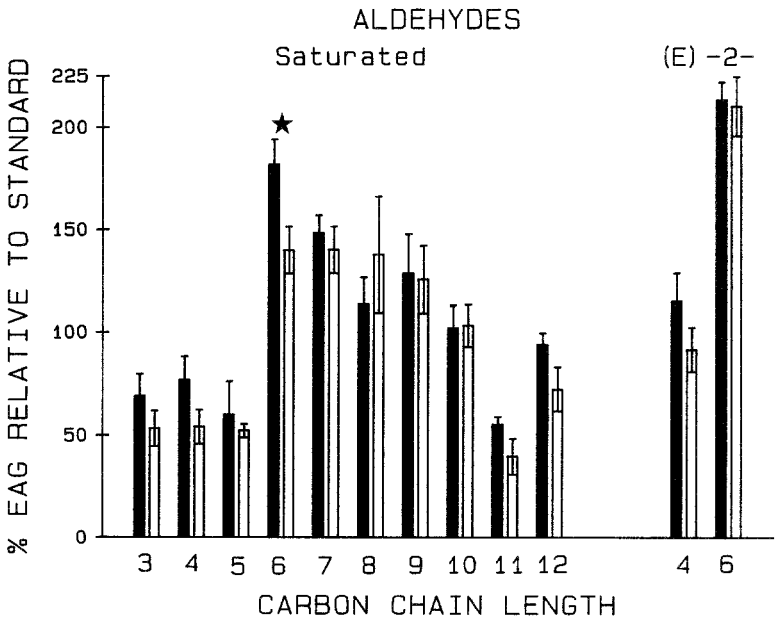

FIG. 5. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of saturated and (*E*)-2-unsaturated aldehydes of various carbon chain lengths. See Figure 1 for further information.

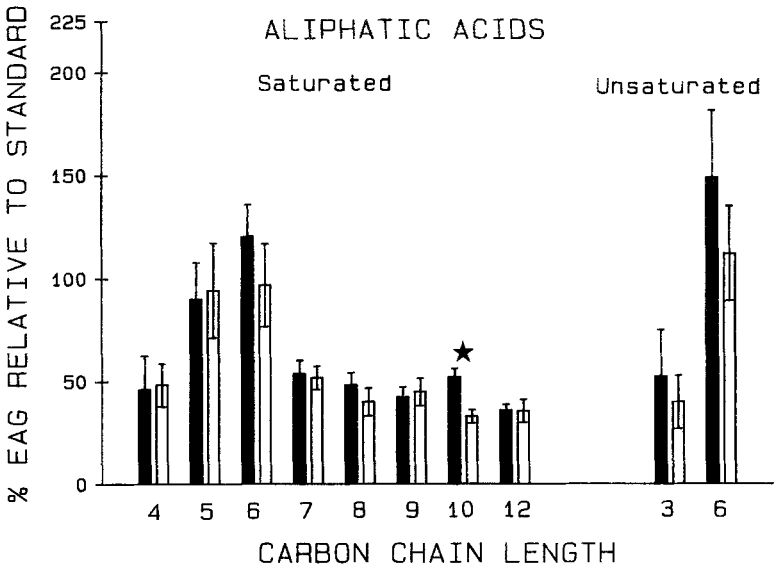

FIG. 6. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of aliphatic acids of various carbon chain lengths and 2-propenoic and (*E*)-2-hexenoic acids. See Figure 1 for further information.

TABLE 2. OCCURRENCE AND MILLIVOLT MAGNITUDE OF NEGATIVE/POSITIVE POLARITY EAGS OBSERVED IN *Ceratitis capitata*

Compound	Sex	Number of positive/negative polarity responses out of five replications[a]			Magnitude of polarity phases[b]	
		ER	−/+	+	Negative ($X \pm$ SE)	Positive ($X \pm$ SE)
Acetic acid	M	0	5	0	−0.46 ± 0.09	+0.93 ± 0.14
	F	1	2	0	−0.19 ± 0.09	+1.48 ± 0.30
Propanoic acid	M	0	3	2	−0.29 ± 0.14	+1.31 ± 0.28
	F	0	2	2	−0.12 ± 0.16	+1.74 ± 0.25
Butanoic acid	M	1	4	0	−0.61 ± 0.08	+1.46 ± 0.25
	F	0	4	0	−0.39 ± 0.28	+1.31 ± 0.27
2-Propenoic acid	M	0	4	0	−0.63 ± 0.28	+1.58 ± 0.17
	F	0	4	0	−0.50 + 0.17	+1.55 ± 0.23
Pentanoic acid	M	2	0	0		
	F	0	0	0		
Hexanoic acid	M	2	0	0		
	F	0	0	0		

[a] ER, early recovery; −/+, negative/positive polarity response patterns; +, positive polarity response patterns; see text for definitions.
[b] Mean millivolt responses for −/+ and + data, exclusive of ER data.

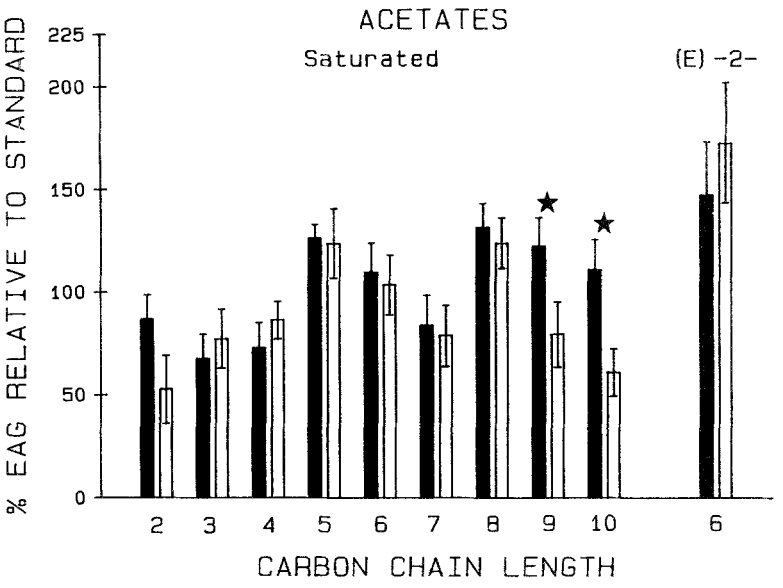

FIG. 7. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of saturated acetates of various carbon chain lengths and the unsaturated (*E*)-2-hexyl acetate. See Figure 1 for further information.

peaked at both C_5 and C_8 (Figure 7). Female antennae were significantly more responsive ($P < 0.05$) than male antennae to both nonyl- and decyl-acetates.

Lactones. The EAGs to a series of lactones did not significantly vary as either (1) side-chain length of the gamma-lactones increased from zero (gamma-butyrolactone) to seven carbons (gamma-undecalactone) except for the greater female responsiveness to gamma-nonalactone (i.e., five-carbon side-chain) or (2) as the lactone ring increased from four (gamma) to five (delta) carbons (Figure 8). Except for gamma-nonalactone, for each lactone tested, male EAGs were slightly, but not significantly, greater than female EAGs.

EAG Response Polarity

The EAGs recorded to the majority of the volatiles tested had purely negative voltage polarities and time-course shapes. Upon odor stimulation, the majority of the recordings typically consisted of a rapid negative voltage deflection that was more or less maintained throughout the odor stimulation duration followed, upon termination of the odor stimulation, by a slower, more gradual (positive polarity) approach or recovery to (but not exceeding) the prestimulation background potential (Figure 9A). However, to specific short chain-length acids alone, a range in degree of biphasic, "negative, then positive polarity" EAGS during odor stimulation was observed (Figure 9B–D). These biphasic

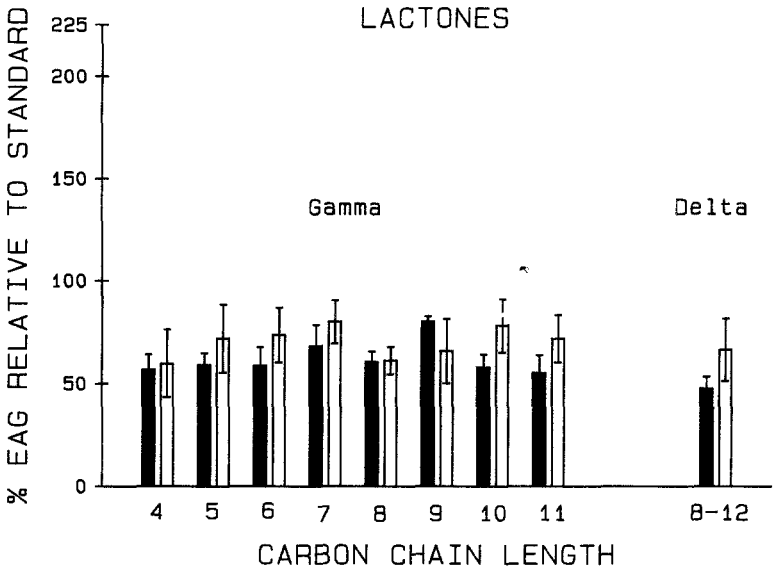

FIG. 8. Mean EAG responses of female (filled bars) and male (blank bars) *C. capitata* to 1-μl doses of 10% solutions (v/v) of gamma-lactones of various carbon chain lengths and a mixture of C_8–C_{12} delta-lactones. See Figure 1 for further information.

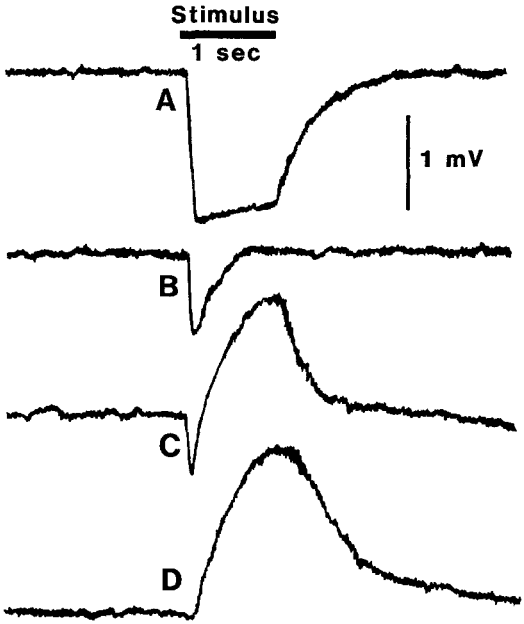

FIG. 9. Reproduction of the four types of EAG polarity and time-course shapes recorded from a single male medfly: (A) typical "negative-polarity" EAG, in this case to hexan-1-ol, (B) "early recovery" EAG to pentanoic acid; (C) biphasic "negative-positive" EAG to butanoic acid; and (D) "positive polarity" EAG to propanoic acid.

EAGs ranged from: (1) "early recovery"; (2) "negative positive" polarity; to (3) an apparently "positive polarity." The "early recovery" was typified by an initial, negative, stimulus-onset response that became spikelike in shape, its recovery to baseline starting immediately during the odor stimulation period and not after its cessation (e.g., 40% of the male's responses to pentanoic and hexanoic acids, Table 2). The "negative/positive" EAG was characterized by the initial negative deflection being reversed by a two to seven times greater positive deflection that greatly overshot the prestimulus baseline during the remainder of the stimulus duration, then, after stimulus termination, the response potential returned to the baseline level [e.g., stimulations by acetic, propanoic, 2-propenoic, and butanoic acids; Table 2]. In the apparently "positive polarity," the response potential never dipped, but rose slowly during stimulation and then dropped to baseline potential after stimulation cessation (e.g., responses of certain individuals to propanoic acid, Table 2).

DISCUSSION

The EAG response is thought to be the expression of generator potentials of many simultaneously stimulated receptor cells with potentially different acceptor specificities (Boeckh et al., 1965; Kaissling, 1971; Schneider, 1969). Further, the negative amplitude of the EAG deflection as been interpreted to be a measure of the relative number of acceptors responding to an odor stimulus (Payne, 1975; Dickens and Payne, 1977). Thus, the EAG results presented here, in general, suggest significant differences in size of acceptor populations for the various odorants and/or odorant classes examined.

Polarity of EAG Responses

Positive polarity and biphasic EAG potentials have been observed before (e.g., Schneider, 1957a, b). Positive polarity EAG responses were elicited by a puff of air during deep ether narcosis in male *Bombyx mori* (L.), while cycloheptanone elicited biphasic "early recovery" waveforms, and air puffs during the beginning phase of ether or chloroform narcosis evoked biphasic negative/positive potentials (Schneider 1957a, b). At the receptor level, Schneider (1969) and others (Boeckh et al., 1965; Kaissling, 1971) have correlated extracellularly recorded, negative-polarity generator potentials (termed depolarizations) with "excitation" (i.e., increased action potential frequency) in receptor neurons, and positive-polarity receptor potentials (termed hyperpolarizations) with "inhibition" (i.e., decreased action potential frequency) in receptor neurons. Boeckh (1962, 1967) and Kaissling (1971) observed positive extracellular DC potentials that they termed "inhibitory potentials" when sensilla basiconica of both the blowfly, *Calliphora erythrocephala*, and the carrion beetles, *Thanatophilus rugosus* and *Necrophorus humator*, were stimulated with propanoic,

butanoic, or pentanoic acids. We found that these same short-chain acids also elicited positive and biphasic EAGs in medfly antennae. However, whether the polarity of EAGs is an accurate representation of receptor potential depolarizations, hyperpolarizations, or the subtractive interaction of the two polarizations must be questioned because of the extreme attenuation inherent in EAG recordings. Further, Kafka (personal communication) and others have suggested that biphasic and positive-polarity EAGs might be recording artifacts attributable to electrode potentials.

Effects on EAG Responsiveness

Sexuality. Although slight differences between males and females in the magnitude of their EAGs to each test odorant were found, in only a few cases were the differences significant. Among the 70 compounds tested, only eight compounds, undecan-1-ol, (*E*)-2-buten-1-ol, (±)-heptan-2-ol, (±)-1-octen-3-ol, hexanal, nonyl acetate, decyl acetate, and decanoic acid, elicited significantly larger EAGs in female antennae than in male antennae. On the other hand, the general similarity in antennal responses between the sexes suggests a common ecological need for host-plant and/or habitat recognition by both sexes. Once alighting on the plant, the sexes might utilize the resource in a similar manner: for example, attaining food (plant sap, nectar, honeydew, protein, etc.); or in a dissimilar manner: for instance, for males as a lekking and courtship site or for females as an ovipositional site.

No sexual dimorphism in EAGs to plant volatiles has been reported for other tephritid fruit flies, e.g., the apple maggot (Fein et al., 1982) and the olive fruit fly (Van Der Pers et al., 1984), or for many other insect species, e.g., *Leptinotarsa decemlineata* (Visser, 1979), *Yponomeuta* species and *Adoxophyes orana* (Van Der Pers, 1981), *Rhynchaenus quercus* (Kozlowski and Visser, 1981), and *Oulema melanopus* and *Pseudaletia unipuncta* (Wellso et al., 1984).

Carbon Chain Length. For four of the five functional-group series tested, both definitive EAG amplitude maxima and antennal responsiveness profiles were affected by variation in carbon-chain length. Overall, it appears that medfly antennae were selectively more responsive to six-carbon and, to a lesser extent, five-, seven-, and eight-carbon, chain lengths for these functional-groups, which are ubiquitous in fruit and leaf volatiles.

The present EAG study is the first to have systematically assessed carbon-chain series on each of these fundamental functional-group classes, while for the most part limited series of only aldehydes and primary alcohols have been tested on other species. Similar to the medfly, the Colorado potato beetle (Visser, 1979) and the oak flea weevil (Kozlowski and Visser, 1981) were found to have peak EAG responses to both C_6 aldehydes and alcohols. Antennal respon-

siveness of the cotton boll weevil, *Anthonomus grandis* (Dickens, 1984), and the cereal aphid, *Sitobion avenae* (Yan and Visser, 1982), peaked at C_6 for the primary alcohol series. However, for aldehydes, the majority of the EAG plant-volatile studies have found that responsiveness peaks at heptanal, with a ranking for most species of C_7, C_8, C_9, and then C_6 [for Diptera: *C. capitata*, *D. oleae*, and *R. cerasi* (Guerin et al., 1983a); *P. rosae* (Guerin and Städler, 1982; Guerin et al., 1983b); and *Delia antiqua* (Guerin and Städler, 1982); for Lepidoptera: *Yponomeuta* spp. (Van Der Pers, 1981) and for Coleoptera: *A. grandis* (Dickens, 1984); and *Trirhabda bacharides* (Dickens and Boldt, 1985)].

Unsaturation. For the limited number of monoenic alcohols, aldehydes, acids, and acetates tested, the unsaturated monoenes elicited equal or larger EAGs than their comparable saturated compounds. Significantly greater responses for both medfly sexes were observed for both (*E*)-2- and (*E*)-3-octen-1-ol over octan-1-ol, (*E*)-2-hexenal and (*E*)-2-butenal over their saturated analogs, and (*E*)-2-hexenyl acetate over hexyl acetate. In addition, female antennae responded significantly greater to (*E*)-2-buten-1-ol than butan-1-ol. In all but one study on EAG response selectivity, the EAG responses to monoenic alcohols, aldehydes, and acetates exceeded those to saturated analogs (see Visser, 1983, 1986). The exception is a study of *D. antigua*, where hexan-1-ol elicited a greater response than the unsaturated isomeric analogs tested (Guerin and Städler, 1982).

Functional Groups. Under our experimental conditions, the greatest antennal responses generally were elicited by aldehyde and alcohol moieties, followed by acetate, lactone, and acid functional groups, as has been found in most species (see above references and Visser 1983, 1986). Furthermore, the monoenic aldehyde, (*E*)-2-hexenal, elicited greater EAGs in medflies than the 2- and 3-position (*E*)- and (*Z*)-hexen-1-ols and other monoenic alcohols, which in turn exceeded the saturated aldehydes and alcohols. The saturated secondary alcohols elicited responses slightly greater than or equal to the saturated primary alcohols.

Thus, carbon-chain length, unsaturation, and type and position of functional groups all have significant effects on the magnitude of the EAG response (i.e., the relative numbers of acceptors responding throughout the antennal olfactory organ) of medflies to odor molecules. The specificities and affinities of the presumed acceptor classes that are present on the medfly antennae are still unknown. However, our preliminary single-cell recordings from various trichoid and basiconic antennal hairs and pegs indicate that receptors are present that are narrowly and specifically tuned to chain length, functional group(s), and chirality of the odor molecules (Dickens et al., unpublished). Of the ca. 30 recordings to date, we have not discovered any receptors with broad responsiveness to various chain lengths of a functional group. However, receptors have been found that are selectively "tuned" to virtually a "single" chain

length for a particular functional group, while responding differentially over a broader range of chain lengths to another functional group. These observations will be explored through further selectivity, sensitivity, and differential adaptability experiments on single receptors.

Green-Leaf Volatiles. The top 16 compounds eliciting the greatest antennal responses of male and female medflies can be ranked as follows: (*E*)-2-hexenal ≥ (*E*)-2-hexen-1-ol ≥ (*Z*)-2-hexen-1-ol ≥ (*E*)-2-octen-1-ol ≥ (*E*)-3-octen-1-ol ≥ (*E*)-2-hexenyl acetate ≥ hexanal > heptan-2-ol ≥ (*E*)-3-hexen-1-ol ≈ (*Z*)-3-hexen-1-ol ≈ hexan-1-ol ≈ heptanal ≈ heptan-1-ol ≈ (*E*)-3-penten-1-ol ≈ (*E*)-2-hexenoic acid ≈ octanal. This ranking is dominated (five of the top 11 compounds) by what have been termed "green odors" or "general green-leaf volatiles" because of the ubiquitous production of these compounds by either intact or, more often, damaged plant leaf parenchyma (see Visser et al., 1979; Buttery, 1981). Produced through the oxidative fragmentation of the plant fatty acids, linoleic and linolenic acids, the following green-leaf volatiles are commonly identified in various ratios, depending on the plant species: hexanal, hexan-1-ol, (*Z*)-3-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexenal, and (*E*)-2-hexen-1-ol.

Along with plant species-specific blends of important discriminatory "key compounds" (often terpenes and their analogs), the green-leaf volatiles dominate the selective EAG responsiveness of insect antennae over aldehydes and alcohols with shorter or longer carbon chain lengths [e.g., carrot fly (Guerin and Visser, 1980); onion and cabbage root flies (Guerin and Städler, 1982); the syrphid fly, *Metasyrphus venablesi* (Hood Henderson and Wellington, 1982); Colorado potato beetle (Visser, 1979); oak flea weevil (Kozlowski and Visser, 1981); cotton boll weevil (Dickens, 1984); the chrysomelid, *T. bacharides* (Dickens and Boldt, 1985); and *Yponomeuta* spp. and *A. orana* (Van Der Pers 1981)]. Thus, as with most species studied electrophysiologically (Visser, 1983, 1986), medflies have antennae "selectively tuned" for reception of green-leaf volatiles. This suggests that medfly antennae have greater populations of acceptors for these six-carbon volatiles. The general receptivity for leaf volatiles by phytophagous insects appears to be comparable, apparently, regardless of the ecological host range of the herbivorous insect, ranging from the near monophagy by *T. bacharides* (a biological control candidate for the weed, *Baccharis halimifolia* L.), to polyphagy by medflies.

Most detailed EAG studies to date have utilized oligophagous insects. In the few studies where kairomonal attractants have been identified or suggested, these are "key compounds" that are relatively unique to the host species or host genus. Such compounds often elicit large EAG responses that significantly exceed the responses to green-leaf volatiles. A well-studied example is the carrot fly where (*E*)-asarone and (*E*)-methylisoeugenol elicit large EAG responses and are attractive in the field (Guerin et al., 1983b). Further, Guerin et al.

(1983b) found that the attraction of *P. rosae* to (*E*)-asarone was synergized by (*E*)-2-hexenal. But in only a few cases have general green leaf volatiles been attractive along (see Visser, 1986; Visser and Ave, 1978). Whether single consitituents of the general green-leaf odor or other fruit odors attract medflies is uncertain. In field tests in Sardinia, Guerin et al. (1983a) found that heptanal attracted predominantly female medflies to sticky traps. However, in field tests in Hawaii, we found no attraction to a total of 21 individual saturated and unsaturated primary alcohols, aldehydes, and acetates (Cunningham et al., unpublished). Thus, questions remain about the effectiveness of the individual components of the green-leaf odor as well as the entire and properly proportioned blend emitted by the plant.

Because of the widespread occurrence of general green-leaf volatiles throughout plants, it is unlikely that their qualitative presence alone could serve to discriminate between host and nonhost plants. On the other hand, the relatively high selectivity and responsiveness of *C. capitata* antennae, and other insect antennae, to the general green-leaf volatiles suggests a large sensory investment in the reception of these compounds that may be adaptive. The reception of green-leaf volatiles may be fundamental to such short- and/or long-range appetitive behaviors that occur on foliage, such as foraging for water, food, and shelter, and the establishment and subsequent attractiveness of lek sites. Because these foraging and lekking behaviors of medflies are readily observed on both host and nonhost plants (Christenson and Foote, 1960; Prokopy and Roitberg, 1984; Prokopy et al., 1986), it is possible that the more universal plant olfactory cues, i.e., the general green-leaf volatiles, might influence these discriminative searching behaviors. The perception of the complex or specific host-plant odors may result in the initiation and maintenance of various searching or foraging behaviors that are mechanistically based on visual optomotor anemotactic and/or phototelotactic orientation (Light, 1986). Such has been hypothesized for the cherry fruit fly (Levinson and Haisch, 1984), the apple maggot (Prokopy, 1986), and the medfly (Féron, 1962; Nakagawa et al., 1978).

A number of additional experiments must be undertaken before determining the applicability of the EAG technique to screen a series of plant volatiles for their potential semiochemical activity with medflies. These additional experiments are presently under investigation (Jang et al., unpublished) and include laboratory and field bioassays, along with comparative EAG studies utilizing known potent male lures (i.e., trimedlure and α-copaene), putative male pheromones (Baker et al., 1985), and the range of volatiles present in specific host fruits.

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